Integrated Water Quality and Aquatic Communities Protocol – Mountain Lakes and Ponds

Standard Operating Procedure (SOP) #11: Amphibian, Invertebrates, and Lake Substrate Walk-around

Draft Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

The purpose of this SOP is to describe the procedures to survey for amphibians, collect invertebrates, and characterize the lake environment. Although these are three disparate activities, all data collection is performed in a single step. Working as a pair, the crew walks around the lake or pond. While one person is actively searching for amphibians and collecting invertebrates, the other member is using a Trimble GeoExplorer Handheld Units (hereafter, "Trimble") to record substrate types, location, and species of amphibians observed, number of amphibians encountered, and location of invertebrate sweeps.

This protocol is presented in four sections: 1) use of Trimble, 2) substrate characterization, 3) amphibian survey methods, and 4) invertebrate collection techniques. A short section at the end is provided to integrate all three.

Responsibilities

It is the responsibility of the Project Lead to make certain field crews have all necessary equipment and field forms to help when collecting data. The Project Lead should work closely with the Data Manager to develop a half-day training session on how to use the data collection equipment. It is the Project Lead and field crew member's responsibility to follow all validation and verification processes when collecting data.

Use of Trimble GPS

The Trimble GPS unit forms the basis of data collection for four components of this protocol; 1) Lake/pond area; 2) type and percentage of near shore lake substrate; 3) numbers, species, and life stages of amphibians; and 4) invertebrate collection locations. Hence, we will be co-collecting habitat type at all locations of amphibians and invertebrate collections. This will allow the calculations of ancillary data, such as the correlation between amphibian abundance and substrates, as well as an accurate assessment of the proportion of substrate types sampled with

the invertebrate collections. **On account of this, crew competence with the Trimble is of critical importance.** Crews must be intimately familiar with the use and trouble shooting of the unit to ensure accurate data collection. Crews must ensure that the Trimble always has an adequate battery charge and that care is taken in the handling of the unit. Note that the collection of lake area is an automatic step and is calculated using GIS applications from the collection of substrate type.

Data Collection

Once the crew is ready to begin collecting data on the perimeter of the lake, amphibians, and invertebrate collection locations, follow the steps listed below. **Start by recording the type of unit used on the datasheet.**

Opening the Project File in ArcPad

A. A few minutes prior to collecting data, turn on the Trimble by clicking the **Green** button on the bottom of the unit.
B. Using a stylus, Tap the word [**GPS**] at the bottom, right side of the screen. A drop down menu should appear.
C. Be patient while ArcPad opens.
D. Although the default on the Trimble will be to automatically have a list of project open, you may need to open the Lakes_Study_XXXX.apm file manually, where XXXX is the current year (e.g., Lakes_Study_2010.apm for the first year of implementation). This file will be set up by the Data Manager prior to the initiation of the field season (SOP #1: Preparations, Equipment, and Safety.
    1. With ArcPad open, in the upper left corner there is a picture of a file. Use the dropdown arrow next to the file to select the "Open Map" option.
    2. Select the "Lakes_Study_XXXX" project.
E. You now need to make certain you have captured enough satellites to provide an accurate location.
    1. Tap the [**GPS Position**] button. Make certain the button looks pressed in.
    2. A small screen will appear showing the satellites. If you see the coordinates in the small screen, you have enough satellites to begin capturing data. If you do not have enough satellites, the Trimble unit will beep repeatedly.
    3. NOTE: If you tap the coordinate, you can change it from lat/long to UTM.
F. When you open the ArcPad project, it is usually zoomed very far out. In the bottom right corner of the screen look at the scale and see what it is set to (e.g., 1:210102).
    1. Double tap the scale and set it to 1:1000.
    2. You should now be able to see the line. Keep in mind you will need to restart the process to collect the perimeter data.
G. Once you have satellite coverage, you can begin to collect data.
H. As you collect data, you may hear a beep and the GPS symbol will change to . This means you are no longer collecting data. Move to a more open area if possible and wait till you get satellite coverage.

Recording the Location of the Lake, Invertebrate Sites, and Amphibians

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A. Make certain you have selected the "Waterbody_Boundary" shapefile by using the edit tool on the drawing toolbar.
B. Once you have enough satellites, using the drop down arrow next to the small dot on the tool bar, select "Polyline" from the list of options.
C. If you are ready to start collecting the perimeter of the lake, click the satellite streaming button and start walking around the lake. REMEMBER to keep the Trimble unit pointed as straight up and down as possible.
D. Walk along the perimeter until you come to: 1) a change in habitat (section 2), 2) an amphibian collection location, or 3) an invertebrate location. Depending on which one of the three options you come to, skip to the appropriate section below.

If You Have Come to a Change in the Habitat

As stated above, you are walking around the lake collecting perimeter (steps A-D above) data and you have come to a change in habitat. At this point, you will record the habitat type you have **just covered, not the new habitat.**

A. When you are at the end of the habitat you are currently walking through, tap the green arrow  at the bottom left side of the screen.
B. This will open the Water Boundary form (Figure 1).
    1. Using the pick lists, complete the form. If the value you need is not in the pick list, you can use the keyboard to enter the data.
        a. Site Name: This is a MANDATORY field.
        b. Primary Habitat: This is a MANDATORY field and should be populated with the most abundant habitat value you **just previously walked through**.
        c. Secondary Habitat: This is a MANDATORY IF APPLICABLE field and should be populated with the second most abundant habitat value you **just previously walked through**. As a rule of thumb, only include a second habitat if there is more than 20% of this habitat type present.
C. Once you have completed the form, tap the green "OK" button at the bottom left side of the screen.
D. On the tool bar, next to the arrow and dot, use the dropdown list to select "Segment Polyline"  .
E. Click the satellite streaming button  and continue walking around the lake.
F. Repeat steps A-H for each habitat type you walk through.

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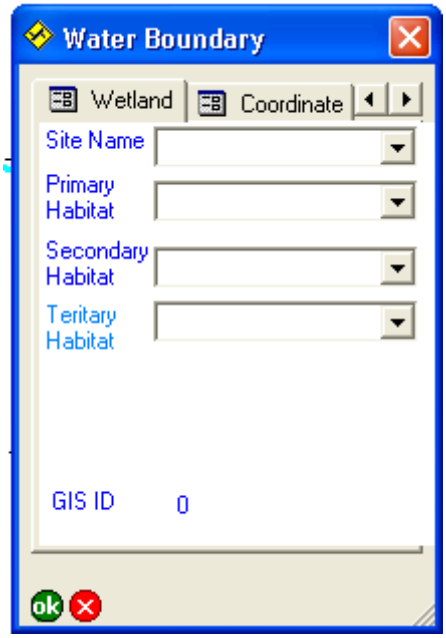

Figure 1. Water Perimeter Data Entry Form on the Trimble GPS Unit.

If You Have Come to a Amphibian or Invertebrate Collection Location

As stated above, you are walking around the lake collecting perimeter data (steps A-C above) and you have come to a location where you see/hear/capture an amphibian or are going to collect invertebrates.

A. Select the "Amphibian" or shapefile using the editing tool under the drawing toolbar.
B. Using the dropdown arrow next to the small dot or polyline (depending on what data was collected previously), select "Point" from the list of options.
   1. Tap the [**Capture GPS Point**] button
   2. This will open the Amphibian form (Figure 2). At the top of the form you will see a count. The GPS will collect 30 point locations and average them for a more accurate location. While the Trimble is collecting the locations, complete the fields in the form.
   3. Enter the following fields.
      a. SITE TYPE: This is a MANDATORY field. Select "Amphibian" from the picklist. NOTE: If this is an invertebrate collection site, select "Invertebrate" and skip to step C below.
      b. NAME: This is a MANDATORY field. Select the scientific name of the species collected. Make your best efforts to determine the genus and the species. If you cannot determine the species just select the genus (e.g., *Rana* sp.). If you cannot determine the genus, select "Unknown Amphibian."
      c. LIFE STAGE: This is a MANDATORY field
         (a) Adult
         (b) Subadult
         (c) Metamorph

(d) Larvae
(e) Egg Sack

d. COLLECTION: This is a MANDATORY field. Select the method the species was observed
(a) Already Dead
(b) Audio
(c) D-Net
(d) Gill Net
(e) Visual

e. COUNT: This is a MANDATORY field. Enter the total number of individuals, estimating as needed.

f. SIZE: Enter the size of the individual in cm, if applicable (e.g., if in hand).

C. Once you have completed this form, tap the green "OK" button in the bottom left corner of the form.

1. Repeat steps 1-4 for each amphibian that is: 1) a different species or 2) is a different size.

D. Click the satellite streaming button and continue walking around the lake collecting perimeter data.

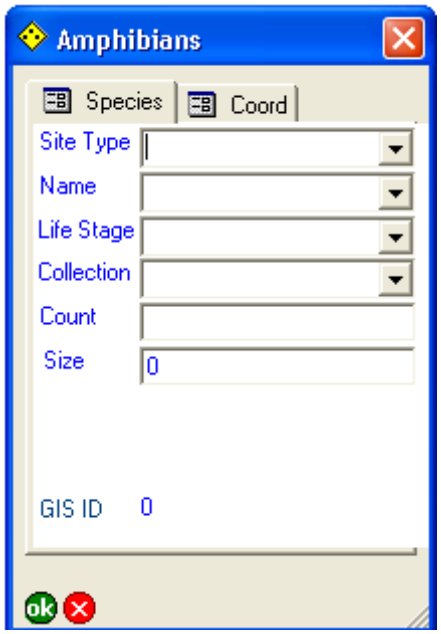

Figure 2. Amphibian and invertebrate data entry form.

Ending the Project

Once you have completed collecting the perimeter and sampling at amphibian and invertebrate locations, you are done using the Trimble and can close out the project and shut off the unit. To do this, complete the following steps.

A. At the top left side of the screen on the toolbar, there is a folder. Using the dropdown arrow, select "Exit." **Note:** When you exit the project, all the data will be saved so you do not have to worry about clicking a save option.

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B. This will close ArcPad. NOTE: If you click the [X] in the upper right corner of the application, it will only minimize the program and not close it. Be sure to close the project when you are done.
C. Tap the large button at the bottom of the screen (just once). This shuts off the unit.

Troubleshooting

Every effort will have been made to set up the Trimble unit and map project before the crew goes into the field. However, situations will occur that cannot be planned for and this section lists some potential issues and the methods on how to correct them.

A. **"NO MATTER WHAT I DO I CANNOT GET SATELLITE COVERAGE."**
   1. The Trimble units are set to a "Productive" setting versus a "Precision" setting to help ensure field crews can quickly gather data. Since this study is sampling lake areas, it is assumed most field sites will be in fairly open habitats with adequate satellite coverage. However if you cannot, complete the following steps.
      a. Compare the settings on the Trimble unit to the settings described in the Settings sections of this SOP. Make sure they are the same. If you still cannot get enough satellite, then go to step b.
      b. Move to an open area, wait for satellite coverage, and then move back to the area you are trying to sample. Continue collecting data but if you lose satellite coverage go to step c.
      c. If you think you cannot get satellite coverage because of the habitat you are sampling in, use the Trimble for those areas of the lake where you can get coverage.

B. **"I HAVE ARCPAD OPEN AND I TAP THE SCREEN BUT ALL IT DOES IT BEEP AT ME."**
   1. Users have the ability to "Lock" ArcPad and occasionally this accidentally occurs. In the upper right corner of the unit is a picture of a lock. Tap it and a message should come up saying "Unlock?" Tap "YES."

C. **"THE POINT AND/OR POLYLINE OPTIONS ARE GREYED OUT SO I CAN NOT COLLECT DATA."**
   1. Each shapefile needs to be in edit mode. Click the pencil on the tool bar. You should see the Amphibians and Waterbodies_Boundary shapefiles. If you do not, complete the following steps.
      a. On the top toolbar, click the image that looks like three sheets stacked on top of each other  . You should see both shapefiles.
      b. In the column with the pencil as the heading, make sure each box is checked.
      c. Click the green "OK" button at the bottom of the screen.

D. **"I KNOW I HAVE DONE EVERYTHING CORRECT BUT AS I WALK THE PERIMETER IT DOES NOT DRAW MY LINE."**
   1. You may be zoomed too far out. In the bottom right corner of the screen look at the scale and see what it is set to (e.g., 1:210102).

2. Double tap the scale and set it to 1:4000.
3. You should now be able to see the line. Keep in mind you will need to restart the process to collect the perimeter data.

E. **"THE PROJECT HAS FROZEN, NOTHING WORKS."**

1. Stop pushing buttons and wait 5 minutes. There may be several things going on and it just needs some time to complete those processes.
2. After waiting 5 minutes, if it has still not worked, you will need to do a hard reset. This should be a LAST RESORT option.
3. Hold the large botton on the Trimble Unit down for 30 seconds and then release it. The unit should turn off.
4. Push the large button again to restart the unit.
5. It will ask you if you want to use the backup copy; say "YES."
6. Reopen your project.

Backing up the Data

Data collected using the Trimble GPS units should be backed up as often as possible. At the end of the day, the GPS data should be backed up onto the laptop computer. This information is repeated in SOP # 12: Post-Site Tasks.

Backing Up Data on the Trimble Units

Make certain you have ActiveSync on the computer where you plan on backing up the data and follow the steps below to back up the data.

1. Connect the Trimble cradle to the computer and place the Trimble unit in the cradle.
2. ActiveSync should start automatically and will let you know when the computer and the Trimble unit are connected.
3. On the computer, open Windows Explorer and look for the Trimble icon that is labeled "Mobile Device" (Figure 3). Double click on the mobile device.
4. Go to the following pathway:
   My Windows Mobile-Based Device\Lakes_Study_XXXX, where XXXX is the year of the study.
5. Right click on the file called "Shapefiles" and select "copy."
6. Go to the location you plan on storing the backup file (on the computer).
7. Right click on the appropriate folder and select "Paste."
8. You have now made a backup of the GPS data.

An alternative, using a "Secure Digital" card (better known as SD cards) can be used by the crew. If the Trimble is equipped with an SD card, transfer the "Shapefiles" folder to the card using the PocketPC mobile Windows Explorer, and then eject the card. Using an external card reader or internal drive (if laptop has one), copy the "Shapefiles" folder to the dated folder (e.g., 20100725) in the GPS_Backup folder. Replace the SD card back in the Trimble.

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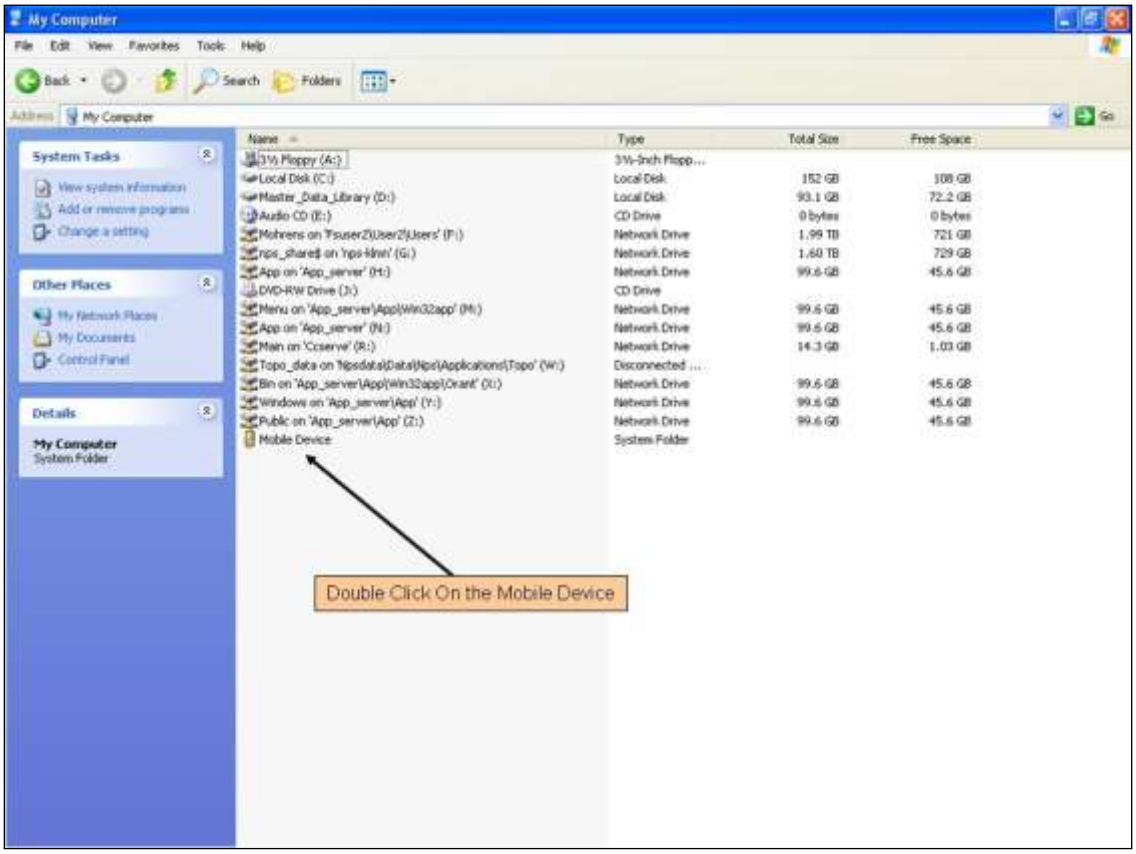

Figure 3. In the Windows Explorer window, users should double click on the mobile device icon to access the Trimble file structure so they can make copies of the GPS data.

Substrate Characterization

Substrate characterization is done by visual observation of the dominant substrate making up the near-shore littoral zone of the lake. This zone is defined by the wetted perimeter to a distance of 3 meters out into the lake. If the slope of the shore is such that substrate 3 meters away is not visible, characterize the habitat based on the most dominant substrate that you can observe. No step by step instructions are included here; this is covered in the basic operation in "Use of Trimble GPS." The following are guidelines in determining how to implement the step by step.

Only characterize the primary dominant substrates. For example, if you are on a beach area with roughly 45% sand, 30% gravel, 15% woody debris, and 10% detritus, enter sand as the primary substrate. The characterization should be based on the superficial areal coverage and not volume or mass. Hence, if emergent vegetation (like water lilies, *Nymphae* sp.) is covering the majority of the habitat, characterize the emergent vegetation as the primary substrate, even though silt or sand maybe more dominant if based on volume.

If the primary and secondary substrate types appear to be in equal proportions, type it as whichever makes the most immediate impression. Alternatively, ask for a second opinion (there will always be two crew members doing this).

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Only start recording a new habitat type in the Trimble when an obvious shift in either primary substrate occurs. An obvious shift is defined by a new substrate that extends a minimum of 2 meters; if the previous substrate type resumes in less than 2 meters, do not type the minor shift in substrates.

Substrate Types

Bedrock
Bedrock is any rock substrate that forms the basal substrate of the lake bottom. Bedrock is generally considered to be greater than 4 m in diameter.

Boulder
Boulders are rock substrates greater than 300 mm (approximately 1 foot) in their longest dimension. Note that they may be submerged or emergent.

Cobble
Cobbles are rock substrates greater than 75 mm (approximately 3 inches) but less than 300 mm in their longest dimension.

Detritus
Detritus is the term used to describe organic debris that is smaller than 25 mm (approximately 1 inch) in their longest dimension. Components will generally be identifiable as small pieces of wood, leaves, or other organic matter that is in the process of decomposition.

Emergent Macrophytes
Emergent macrophytes are plants (or macroalgae) that emerge from the water. This category is also used for macrophytes that are on the water surface (e.g., water lilies).

Gravel
Gravel is composed of rock substrate particles greater than 2 mm but less than 75 mm in their longest dimension.

Sand
Sand is composed of rock substrate particles less than 2 mm in their longest dimension.

Fines
“Fines” is a mix of inorganic and organic particles of a very fine particles size (generally less than 0.5 mm). Unlike sand, which will feel gritty between one’s fingers, fines will feel soft and fine, like baking flour. Fines may be characterized by dark organic particles. Fines without any organic debris would be a sediment like clay (or “muck”). The intricacies of determining the amount of organic component to silt necessitate the lumping of clay into the “fine” category.

Submergent Macrophytes
Submergent macrophytes are plants (or macroalgae) that do not emerge through the water surface.

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Woody Debris

Woody debris is organic substrates from terrestrial plants (e.g., wood branches, fallen logs, rotting rootwads, etc.) that make up the lake bottom. They will generally be large logs and branches. If the longest dimension is over 25 mm, characterize as woody debris. If under, then categorize as detritus. Note that woody debris may often be floating.

Amphibian Survey

The purpose of the amphibian surveys is to develop Presence/Not Detected lists for each lake or pond. Qualitative estimates of abundance are a secondary purpose. However, the survey techniques are oriented towards maximizing detection and not strict abundance estimates. Amphibian surveys are accomplished using Visual Encounter Surveys (VES) and targeted searches. As the crew member recording habitat data walks the lake wetted perimeter, the other walks within the lake or pond body and continuously searches for visual or aural signs of amphibians. As amphibians are observed, the species or genus encountered, life stage, and the approximate number seen are recorded as in Section 1 on Trimble use.

In addition to the continuous search, a minimum of 15 intensive searches throughout the lake perimeter should be conducted. The intensive search is composed of turning over rocks and logs and searching through macrophytes. Amphibians encountered should be recorded in the Trimble as described above.

Intensive searches should be conducted at equal spacing around the lake shore. An initial estimate of the circumference of the lake should be taken prior to the initiation of the walk around. Divide this estimate by 15 to estimate the approximate distance between intensive searches. Although an estimate of distance is used, the actual searches should be done on the most likely habitat for amphibians, even if the most likely substrate is not immediately in this location. The crew member in the water should spend several minutes turning over substrate, actively searching for amphibians. When found, the amphibian species, life stage, and numbers are recorded in the Trimble as described above.

Amphibian Walk-Around Procedure step by step

A. Start by making a visual estimate of the approximate perimeter, in meters. This estimate does not have to be precise; it is only an estimate. Divide your estimate by 15 to estimate 15 equally spaced sampling location. This distance will form your basis for intensive search locations. Note: if this lake is an index lake and has been sampled previously, the recorded actual perimeter should be available to guide the spacing of sampling locations.
B. Proceed to walk around the lake. One crew member walks the wetted perimeter and records substrate type (as described above) while the other crew member wades in the water.
C. As amphibians are encountered in the visual encounter survey, the crew member walking the wetted perimeter will **record the species, life stage, and approximate number in the Trimble** (as described above). It is the responsibility of the crew member in the water to visually search for amphibians, as the other crew member is focusing on perimeter and substrate typing and data entry.

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D. While walking, pace off the approximate distance calculated in A. above. When arriving at this spot, look for the nearest (within 10 m) likely habitat for amphibians. The most likely habitat will be areas with structure (e.g., logs, rocks, macrophytes). At this location, conduct an intensive search.

1. Turn over likely structures (logs and such) and search for amphibians. The intensive search should be done over a minimum of 4 m^2 for at least 3 minutes.
2. If the intensive search site is not near any likely habitat, the crew should ensure that there are no amphibians in this area and move on. Note that if the substrate is a sandy beach, the time required may be much less than 3 minutes. Use the 3 minute rule only for complex habitats.
3. Do not stop searching if you find a single amphibian species. Continue searching for additional species until the entire square area is searched.
4. The crew member walking the perimeter should **record the data** in the Trimble (as described above).
5. Note that data from visual surveys and intensive searches are entered identically and that no distinction is made between occurrences.

E. If necessary, individuals may be captured for species verification. The following handling procedures should be followed (See also: American Society of Ichthyologists and Herpetologists, 2004. Guidelines for use of live amphibians and reptiles in field and laboratory research, Second edition. Available at http://www.asih.org/files/hacc-final.pdf.)

1. Capture techniques should be as minimally invasive as possible.
2. Minimize handling time.
3. Always wear a protective layer (e.g., neoprene or latex gloves). This will prevent introducing toxins (e.g., insect repellent or sunscreen) through the amphibian skin. It will also prevent tetrodotoxin in *Taricha granulosa*, the Roughskin Newt, from affecting the handler.
4. Always handle the amphibian with wet hands. If hands dry out during handling, rewet your hands.
5. Release the specimen in the exact place where the organism was captured.

F. As the crew maps the wetted perimeter, the crew should reassess the distance between the intensive search areas so that the crew does not return to the starting point before finishing 15 intensive searches.

Amphibian Disease Protocol

Crews should be on the lookout for signs of amphibian diseases. There are two primary diseases of concern for the amphibians of Lassen Volcanic, Crater Lake, and Redwood National Parks: (1) *Batrachochytrium dendrobatidis* (*Bd* for short, also known as "Chytrid Fungus"), and (2) Ranavirus.

For either disease, the main indications that will be observable to field crews will be:

- Massive die-offs – Large numbers of dead adults, larvae, or metamorphs may indicate presence of either disease.
- Lethargic individuals – Amphibians of any life stage or species that exhibit slow responses to capture attempts (or no response) may indicate disease. Do not handle these individuals without gloves!

- Abnormal morphology – The presence of additional limbs, discolored limbs, or peeling of skin may indicate disease. Swelling and redspots in the ventral region can also indicate internal bleeding consistent with diseases.

The ability to routinely test individuals for either disease is beyond the logistical and financial ability of this protocol. For example, the cost to routinely test all frog species for *Bd* and the time for crews to immediately ship specimens off to analytical labs is prohibitive.

Instead, upon identification of a possible disease outbreak, the Field Crew Leader should inform the Project Lead as soon as possible, preferably the same day as the identification. The Project Lead should consult with the following entities:

USGS National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711-6223.
Phone: (608) 270-2400
Online at: http://www.nwhc.usgs.gov/

And

National Park Service
Biological Resource Management Division – Wildlife Health
1201 Oakridge Drive, Suite 200
Fort Collins, CO 80525
Phone: (970) 267-2162
Online at: http://www.nature.nps.gov/biology/

In providing diagnoses, the USGS National Wildlife Health Center will be able to instruct the Project Lead on specimen collection and shipping procedures to ensure quality specimens for diagnoses. These methods may change from year to year and from likely diseases. Live specimens may be preferred but will require same-day shipping using overnight services.

The National Park Service – Biological Resource Management Division similarly maintains wildlife health veterinarians who may assist the Project Lead in obtaining technical help with the possible disease.

Disinfection after every site is a necessary step to be taken after every habitat (SOP# 12: Post-Site Tasks). **HENCE, DISINFECTION TO PREVENT THE ADDITIONAL SPREAD IS DONE REGARDLESS OF WHETHER OR NOT THE CREW OBSERVES ANY SIGNS OF DISEASE**. The disease may be dormant or asymptomatic in the amphibian populations; failure to disinfect may spread the disease further.

Invertebrate Collection Procedures

Invertebrate collections are done by net sweeps in the littoral zone of each lake. The nature of a lentic habitat necessitates a semi-quantitative collection procedure, described herein and adapted

from Knapp et al. (2005). Strictly quantitative collections are prohibited due to the logistics of heavy corers and motile nektonic invertebrates. Plus, the wide dispersion of lentic invertebrates dictates a large total area being sampled to adequately sample the assemblage.

Collections are comprised of 15 "standard sweeps." A single standard sweep is done by rapidly dragging a D-net (304 mm wide, 0.5 mm mesh size) over a 1 m stretch of substrate. The net should follow the bottom contours and only sample the water column, epibenthos, and surficial sediments. The sweep is followed immediately by a sweep in the opposite direction so that the habitat sampled in the first sweep is resampled. This method is semi-quantitative because the 1 m stretch is visualized and not measured. A 1 m increment marked on the net handle is used to guide the crew member in this visualization so that the sweep length closely approximates 1 m.

After the sweep, the collected material is deposited in a bucket for processing and preserving in 95% Ethanol. If too much material is collected over the 15 sweeps to fit in a volume of 2 L, the collected material can be split in the field. This is done by mixing the material in the bucket so that it is homogenous, pouring the debris on a 500 µm sieve, and dividing the material into two sections. A coin flip determines which half to retain, and which to dispose of. The material can be further split, if necessary. The material is then transferred to 500 ml vials (up to four if necessary), labeled with a weather-proof paper with pencil added to each vial, and preserved with 95% Ethanol. Note that the water content is high in the debris, so that the final concentration will be closer to 75-80% Ethanol.

Invertebrate Collection Step by Step

A. Prior to beginning the collection, label the collection vials with the pre-made labels SOP #9: Water Sample Filtration and Handling). Attempting to attach the labels to the vials after processing will result in difficulty in securely attaching the labels.
B. As with the amphibian intensive search sites (Step A), make a visual estimate of the lake perimeter. This will indicate the spacing between invertebrate collection sites. Note that unlike the amphibian intensive search sites, the invertebrate collection occurs at the exact site (i.e., there is NO moving the location to a "better" habitat). The invertebrate collection area should be offset from the amphibian intensive search sites.
C. Upon arriving at an invertebrate collection site, visualize the area to be swept. Using the 1 m mark on the net handle, make a mental note of the approximate length of 1 m. Sweep the net, by lightly dragging the net on through the surficial sediments in a 1 m length of habitat parallel to the lake shore. The net should be swept with a rapid movement; this will increase the likelihood of capturing fast moving, swimming invertebrates that may exhibit avoidance behavior.
D. **Immediately**, at the end of the 1 m sweep, twist the net 180 degrees, so that the net is turned around (but without flushing the previously collected debris). Again, **immediately** sweep the net back over the 1 m sweep length, so that any material disturbed into the water column is collected in the net.
E. Deposit the collected debris (with invertebrates) into a collapsible bucket.
F. While the crew member is in the water, **the crew member who is walking the wetted perimeter enters the location of the invertebrate collection into the Trimble** (as above in Section 1).

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G. Continue with the walk-around, recording the substrates and amphibian visual search as in the above sections.
H. Repeat steps A – E at the remaining 14 invertebrate collection sites, adding debris to the collapsible bucket. If required, two or more buckets can be used to collect the debris.
I. After collecting in 15 sites, process the collected debris by transferring to containers.
    1. While the debris is in the bucket, remove large particles of debris (pinecones, sticks, etc.). Before removal, rinse with water to ensure that no invertebrates are hanging on. Dump removed particles on the shore or back into the lake. Do not spend a lot of time on this; a maximum of 15 minutes is recommended. If the other crew member has other tasks remaining, more than 15 minutes can be spent on this at the discretion of the Crew Leader.
    2. If the remaining collected material is greater than 2 L, it is necessary to do a "field" split.
        a. Pour the debris onto the 500 µm sieve. It is best to work in moderate portions of debris; not all the debris must be split at once.
        b. Stir or agitate the debris so that it the debris is homogenous in the sieve.
        c. Use the splitter (a length of lightweight steel plate that matches the interior diameter of the sieve) to split the debris into two equal portions.
        d. Using a coin flip or other random number generator, discard one half of the debris back into the lake. Note that this portion may contain hundreds of invertebrates, so it is best to return it to the lake or pond.
        e. Repeat a-d until the entire original collection has been split.
        f. If the sample is still greater than 2 L, repeat a-d again (effectively so that a 25% portion of the original sample is retained).
    3. Once the sample has been reduced to fit within a 2 L volume, transfer the material to the collection vials.
    4. The vials may be filled to the top, but do not compact the material; Ethanol must be able to penetrate the material for preservation.
    5. Fill the vials with 95% Ethanol, so that Ethanol covers the debris.
    6. Fill out a paper label (on Rite-in-the-Rain paper) with a pencil. **Be sure to indicate the total number of vials and the amount of field split.**
        a. Write "1 of 4;" "2 of 4," etc. Do not use short hand, as fractions could be confused with percentages (e.g., "1/4" is a fraction [25%], and could be confused with the split count). Use % to indicate the amount of field split (one complete split = 50%; two splits = 25%; three splits = 12.5%; four splits = 6.25%). If there were no splits, indicate with a 100%.
        b. Record the number of vials and the split percentage on the field data sheet.